

- a) applying to the cells a thiazine dye to stain the cells;
- b) removing excess dye from the cells;
- c) generating a reflected spectrum by irradiating the stained cells with light to induce photo oxidation of the biological stain or dye; and
- d) simultaneously monitoring the reflected spectrum of the stained or dyed cells during irradiation.

Please cancel claims 3 and 15, without prejudice.

STATUS OF THE CLAIMS

Claims 1, 3, 5, 7-13, 15 and 17-19 are pending prior to this Amendment.

Claims 3 and 15 are being cancelled by this amendment.

Claims 1, 12 and 13 are being amended.

Claims 1, 5, 7-13 and 17-19 are pending following this amendment.

REMARKS

Applicant is amending claims 1, 12 and 13 to recite applying to the tissue or cells "a thiazine dye". This amendment is being made solely to particularly claim a preferred embodiment of Applicant's invention. This amendment introduces no new matter, and is supported in the specification as filed, *inter alia*, on page 20, lines 5-8.

Applicant is amending claims 1, 12 and 13 to recite the step of removing excess dye from the tissue or cells. This amendment is being made to more particularly point out and distinctly claim Applicant's invention, and is supported in the specification as filed, *inter alia*, on page 21, lines 24-26.

Claims 12 and 13 are also being amended to correct punctuation.

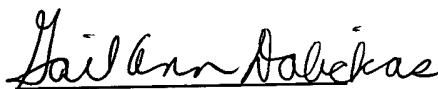
The present claims are directed to methods for diagnosing disease states in tissue or cells, and to methods for cytotoxic destruction of dysplastic, pre-cancerous or cancerous cells. The methods include the application to the tissue or cells a thiazine dye. Thiazine dyes are metachromatic; i.e. they have the capacity to stain different elements of a cell or tissue in different colors or shades. This property enables the use of thiazine dyes to diagnose disease states in which the interaction between such dyes and cells or tissue is affected, directly or indirectly, by the effects of the disease state on the cells or tissue. While in vivo staining of cells and tissues is known, conventional staining techniques do not distinguish between normal cellular repair processes and metaplastic states. Rather, staining has been used to locate diseased tissue or cells but additional histochemical techniques have been required for diagnosing disease states.

While prior art methods rely on distinguishing between stained and unstained features of cells or tissues, the present method utilizes a library of spectra with which a diagnostic spectrum can be compared, allowing a differentiation between diseased tissue and tissue undergoing normal repair. Accordingly, removal of excess dye from cells or tissue prior to obtaining a spectrum is required. Moreover, background spectra of the cells or tissue, taken before application of a dye, provides improved diagnostic accuracy. It is respectfully submitted that the prior art does not teach or suggest these features.

Applicant respectfully submits that claims 1, 5, 7-13 and 17-19 are patentable over the prior art, and prompt allowance is earnestly solicited.

Respectfully submitted,

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A handwritten signature in cursive script, reading "Gail Ann Dalickas".

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Version of claims with markings to show changes made:

1. A method for diagnosing a disease state in situ in biological tissue or cells of a living organism comprising:

- a) applying to the tissue or cells a [biological stain or dye or a combination of biological stains and dyes] thiazine dye;
- b) removing excess dye from the tissue or cells;
- [b)] c) generating a reflected light spectrum from the tissue or cells by illuminating the stained tissue or cells with light;
- [c)] d) directing the reflected light spectrum to a spectrometer;
- [d)] e) comparing the reflected spectrum of the stained tissue or cells with a library of previously obtained spectra; and
- [e)] f) correlating the reflected light spectrum with a disease state, whereby an in situ diagnosis of a disease state is made.

3. A method as in Claim 1 where the biological stain or dye or combination of biological stains or dyes comprises a metachromatic biological stain or dye.

5. A method as in Claim 1 wherein said comparing comprises the use of a digital microprocessor.

7. A method as in Claim 1 wherein the tissues or cells are thought to be diseased or metaplastic.

9. A method as in Claim 1 where the reflected light spectrum is measured and recorded, and said measuring comprises the use of a photometer and one or more light filters.

10. A method as in Claim 1 wherein the tissues or cells are of at least one organ selected from the skin, cervix, vagina, mouth, colon, esophagus or internal organs.

11. A method as in Claim 1 wherein, prior to said comparing step, a reflected light spectrum from unstained tissue or cells is subtracted from the spectrum of the stained tissue or cells.

12. A method for diagnosing a disease state in situ in biological tissue or cells of a living organism comprising:

a) applying to the tissue or cells a [photo-reactive biological stain or dye or a combination of photo-reactive biological stains and dyes] thiazine dye to stain the tissue or cells;

b) removing excess dye from the tissue or cells;

[b)] c) generating a reflected light spectrum of the [photoreactive biological stain or] thiazine dye applied to the stained tissue or cells by illuminating the stained tissue or cells with light while simultaneously measuring and recording the reflected light spectrum of the [photoreactive biological stain or] dye [applied to the stained tissue or cells with a cytochemical or histochemical property of the tissue or cells.] ;

[c)] d) correlating the reflected light spectrum;

[d)] e) directing the reflected light spectrum to a spectrometer;

[e)] f) comparing the reflected spectrum of the stained or dyed tissue or cells with a library of previously obtained spectra; and

[f)] g) correlating the reflected light spectrum with a disease state, whereby an in situ diagnosis of a disease state is made.

13. A method for the cytotoxic destruction of dysplastic, pre-cancerous or cancerous cells comprising:

- a) applying to the cells a [biological stain or dye or a combination of biological stains and dyes] thiazine dye to stain the cells
- b) removing excess dye from the cells;
- [b)] c) generating a reflected spectrum by irradiating the stained cells with light to induce photo oxidation of the biological stain or dye; and[;]
- [c)] d) simultaneously monitoring the reflected spectrum of the stained or dyed cells during irradiation.

15. A method as in Claim 13 where the biological stain or dye or combination or biological stains and dyes comprises a metachromatic biological stain or dye.

17. A method as in Claim 13 wherein said monitoring comprises measuring the reflected spectrum, using a spectrometer able to measure light having a wavelength from 200 to 1100 nanometers.

18. A method as in Claim 13 where the reflected light spectrum is measured and recorded, and said measuring comprises the use of a photometer and one or more light filters.

19. A method as in Claim 13 where the tissues or cells are selected from the group consisting of the skin, cervix, vagina, mouth, colon, esophagus, and internal organs.